Amendments to the Claims

This listing of claims will replace all prior versions, and listing, of claims in the application.

1. **(Currently Amended)** A method for preparing a polyfunctionalized peptide comprising a peptidic backbone made up of four or more amino acids wherein two or more non-adjacent amino acids are independently substituted with a moiety having the structure:

$$A$$
 L^1 ξ

with the proviso that the peptide sequence between any two consecutive, non-adjacent, amino acids bearing a Λ -L¹-moiety comprises at least one cysteine residue;

wherein the method comprises a step of:

reacting a peptide acyl donor comprising a peptidic backbone made up of two or more amino acids wherein said peptide acyl donor has the structure:

with a peptide amine acceptor having the structure:

R^{S1}S
$$\begin{pmatrix} A_2 \\ L^1 \end{pmatrix}_{k2}$$
 Peptide Backbone \mathbb{R}^{x2}

under suitable reducing reaction conditions employing an excess of a reducing agent to effect ligation;

wherein k1 and k2 are independently integers between 1 and about 20;

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each occurrence of A, A_1 and A_2 is independently an aliphatic, heteroaliphatic, aromatic, heteroaromatic, aryl, or heteroaryl or a pharmaceutically useful group or entity;

R^{S1} is a sulfide protecting group;

R^{X0} is a <u>disulfide-substituted aryl moiety</u> group such that the moiety - C(=O)OR^{X0}-can be made to undergo ligation with the peptide amine acceptor;

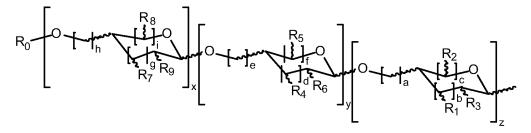
each occurrence of L¹ is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety;

R^{X1} is hydrogen, alkyl, acyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

 R^{X2} is $-OR^{X2a}$ or $-NR^{X2b}R^{X2c}$, wherein R^{X2a} is hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a carboxylic acid protecting group, an amino acid or a proctected amino acid; and R^{X2b} and R^{X2c} are independently hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid.

2. (Canceled)

- 3. **(Currently Amended)** The method of claim 1, wherein each occurrence of A, A1 and A2 is independently a biomolecule, a small molecule, a macromolecule or a diagnostic label.
- 4. **(Currently Amended)** The method of claim 1, wherein each occurrence of A, A1 and A2 is independently a carbohydrate determinant having the structure:



wherein a, b, c, d, e, f, g, h, i, x, y and z are independently 0, 1, 2 or 3, with the proviso that the x, y and z bracketed structures represent furanose or pyranose moieties and the sum of b and c is 1 or 2, the sum of d and f is 1 or 2, and the sum of g and i is 1 or 2, and with the proviso

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that x, y and z are not simultaneously 0; wherein R₀ is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ and R₉ is independently hydrogen, OH, ORⁱ, NHRⁱ, NHCORⁱ, F, CH₂OH, CH₂ORⁱ, a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of Rⁱ is independently hydrogen, CHO, COORⁱⁱ, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group or a saccharide moiety having the structure:

wherein Y and Z are independently NH or O; wherein k, l, r, s, t, u, v and w are each independently 0, 1 or 2; with the proviso that the v and w bracketed structures represent furanose or pyranose moieties and the sum of l and k is 1 or 2, and the sum of s and u is 1 or 2, and with the proviso that v and w are not simultaneously 0; wherein R'₀ is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R₁₀, R₁₁, R₁₂, R₁₃, R₁₄ and R₁₅ is independently hydrogen, OH, ORⁱⁱⁱ, NHRⁱⁱⁱ, NHCORⁱⁱⁱ, F, CH₂OH, CH₂ORⁱⁱⁱ, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R₁₆ is hydrogen, COOH, COORⁱⁱ, CONHRⁱⁱ, a substituted or unsubstituted linear or branched chain alkyl or aryl group; wherein each occurrence of Rⁱⁱⁱ is hydrogen, CHO, COOR^{iv}, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group; and wherein each occurrence of Rⁱⁱⁱ and R^{iv} are each independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group.

5. **(Withdrawn)** The method of claim 1, wherein each occurrence of L^1 is independently – $O-(CH_2)_n$ -, wherein n is 0-9, or a glycoside-containing moiety.

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6. **(Withdrawn)** The method of claim 1, wherein L^1 is -O- $(CH_2)_n$ - CH_2 - and two or more non-adjacent amino acids is/are independently substituted with a moiety having the structure:

$$A$$
 CH_2 S

wherein each occurrence of n is independently 0-8.

- 7. **(Currently Amended)** The method of claim 1, wherein each occurrence of A, A1 and A2 is independently selected from the group consisting of Globo-H, fucosyl GM1, KH-1, glycophorin, STN, STn, (2,3)ST, Le^y, Le^x, N3, Tn, 2,6-STn, 2,6-ST, Gb3 and TF.
- 8. **(Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

$$\mathbb{R}^{X1} \left[\begin{array}{c} A_1 \\ A_2 \\ A_3 \\ A_4 \\ A_5 \\ A_7 \\ A_8 \\ A_8 \\ A_9 \\ A_9 \\ A_{11} \\ A_{11} \\ A_{12} \\ A_{13} \\ A_{14} \\ A_{15} \\ A_{1$$

wherein s1 and s2 are independently an integer from 1 to about 20;

t1, t2 and t3 are each independently an integer;

R^{X1} is hydrogen, alkyl, acyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

 R^{X2} is $-OR^{X2a}$ or $-NR^{X2b}R^{X2c}$, wherein R^{X2a} is hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a carboxylic acid protecting group, an amino acid or a proctected amino acid; and R^{X2b} and R^{X2c} are independently hydrogen, alkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), a nitrogen protecting group, an amino acid or a proctected amino acid;

R^{P1}, R^{P2} and R^{P3} are independently H, alkyl, heteroalkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), or a natural or non-natural amino acid side chain;

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each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety;

 A_1 and A_2 are each independently an aliphatic, heteroaliphatic, aromatic, heteroaromatic, aryl, <u>or</u> heteroaryl or a pharmaceutically useful group or entity; and

at least one occurrence of the bracketed structure t2 is a cysteine residue or protected cysteine residue;

and the method comprises a step of:

reacting a peptide acyl donor having the structure:

$$R^{\times 1} = \begin{bmatrix} H & O \\ S & D \\ R^{P1} \end{bmatrix}_{t1} \begin{bmatrix} H & O \\ H & D \\ H & O \end{bmatrix}_{s1} \begin{bmatrix} H & O \\ R^{P2} & D \\ R^{P2} & D \end{bmatrix}_{t} OR^{\times 0}$$

with a peptide amine acceptor having the structure:

$$\begin{array}{c|c} R^{S1}S \\ H_2N \end{array} \begin{array}{c} A_2 \\ H_2N \end{array} \begin{array}{c} A_2 \\ R^{P2} \\ R^{P2} \end{array} \begin{array}{c} A_1 \\ R^{P2} \\ R^{P3} \end{array} \begin{array}{c} A_2 \\ R^{P3} \end{array} \begin{array}{c} A_2 \\ R^{P3} \end{array}$$

under suitable reducing reaction conditions employing an excess of a reducing agent to effect ligation;

wherein the sum t+t' equals (t2)+1; R^{S1} is a sulfide protecting group; and R^{X0} is a group such that the moiety $-C(-O)OR^{X0}$ can be made to undergo ligation with the glycopeptide amine acceptor.

9. **(Currently Amended)** The method of claim 8, wherein the step of reacting the peptide acyl donor with the peptide amine acceptor is repeated a desired number of times, to prepare a polyfunctionalized peptide having the structure:

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$$\mathbb{R}^{X1} = \mathbb{R}^{P0} = \mathbb{R}^{P0} = \mathbb{R}^{P0} = \mathbb{R}^{P1} = \mathbb{R}$$

wherein R^{X1} and R^{X2} are as defined in claim 8;

each occurrence of A may be the same or different and may be as defined for A_1 and A_2 in claim 8;

each occurrence of R^{P1} may be the same or different and may be as defined for R^{P1} and R^{P2} in claim 8;

q is an integer greater than or equal to 2;

each occurrence of s is independently an integer from 1 to about 20;

each occurrence of t is independently an integer;

t0 is an integer; and

each occurrence of R^{P0} is independently H, alkyl, heteroalkyl, aromatic, heteroaromatic, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), or a natural or non-natural amino acid side chain.

- 10. (Original) The method of claim 9, wherein q is an integer between 2 and about 5.
- 11. **(Original)** The method of claim 9, wherein q is 2.
- 12. **(Original)** The method of claim 9, wherein the sum s+t is between about 2 and about 6.
- 13. (Original) The method of claim 9, wherein t0 is an integer from 0 to about 20.
- 14. (Original) The method of claim 9, wherein R^{X1} is hydrogen, Fmoc or Ac.
- 15. (Original) The method of claim 9, wherein R^{X2} is NH₂.

16. (Cancelled)

17. (Original) The method of claim 9, wherein R^{X0} has the structure:

wherein R is an aliphatic, heteroaliphatic, aromatic or heteroaromatic moiety.

18. (Original) The method of claim 17, wherein R^{X0} has the structure:

wherein R is lower alkyl.

- 19. **(Original)** The method of claim 18, wherein R is ethyl.
- 20. (Original) The method of claim 9, wherein R^{S1} is -StBu.
- 21. **(Currently Amended)** The method of claim 9, wherein in the step of reacting the peptide acyl donor having the structure:

$$R^{X1} = \begin{bmatrix} H & O \\ P^{1} & H \\ R^{P1} & H \end{bmatrix}$$

$$R^{X1} = \begin{bmatrix} H & O \\ R^{P1} & H \\ R^{P1} & H \end{bmatrix}$$

$$R^{X1} = \begin{bmatrix} H & O \\ R^{P2} & H \\ R^{P2} & H \end{bmatrix}$$

$$R^{X1} = \begin{bmatrix} H & O \\ R^{P2} & H \\ R^{P2} & H \end{bmatrix}$$

with the peptide amine acceptor under suitable conditions to effect ligation, an intermediate having the following structure is formed in situ:

$$\mathbb{R}^{X1} \left[\begin{array}{c} H \\ N \\ R^{P1} \end{array} \right]_{t1} \left[\begin{array}{c} H \\ N \\ H \end{array} \right]_{0} \left[\begin{array}{c} H \\ N \\ R^{P2} \end{array} \right]_{t} \mathbb{S}^{R^{X0a}}$$

wherein R^{X0a} is an oxygen-substituted aryl moiety.

- 22. **(Currently Amended)** The method of claim 21, wherein the suitable conditions to effect ligation comprise reducing agent is 2-mercaptoethanesulfonic acid, sodium salt MESNa.
- 23. **(Currently Amended)** The method of claim 9, wherein in the peptide acyl donor having has the structure:

the amino acyl residue directly attached to $-OR^{X0}$ is phenylalanine.

24. (Withdrawn/Currently Amended) The method of claim 1, wherein when at least one occurrence of A (or A_1 and/or A_2 , as further defined for A) is a carbohydrate domain, and some

or all of carbohydrate domains are O-linked to the peptide backbone.

25. (Currently Amended) The method of claim 1, wherein when at least one occurrence of

A (or A₁ and/or A₂, as further defined for A) is a carbohydrate domain, and some or all of

carbohydrate domains are N-linked to the peptide backbone.

26. (Withdrawn/Currently Amended) The method of claim 1, wherein the

polyfunctionalized peptide is symmetrical.

27. (Currently Amended) The method of claim 1, wherein the polyfunctionalized peptide is

nonsymmetrical.

28. (Withdrawn/Currently Amended) The method of claim 1, further comprising a step of

conjugating the polyfunctionalized peptide to an immunogenic carrier.

29. (Withdrawn) The method of claim 28, wherein the carrier is a protein, a peptide or a

lipid.

30. (Withdrawn) The method of claim 28, wherein the carrier is Bovine Serum Albumin

(BSA), Keyhole Limpet Hemocyanin (KLH) or polylysine.

31. (Withdrawn) The method of claim 28, wherein the carrier is a lipid carrier having the

structure:

wherein m, n and p are each independently integers between about 8 and 20; and $R_{\rm V}$ is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl.

- 32. (Withdrawn) The method of claim 31, wherein m', n' and p' are each 14.
- 33. **(Withdrawn/Currently Amended)** The method of claim 28, wherein the carrier is linked to the polyfunctionalized peptide through a crosslinker.
- 34. **(Withdrawn/Currently Amended)** The method of claim 33, wherein the crosslinker is a fragment having the structure:

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a suitable functionality on the polyfunctionalized peptide.

35. **(Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

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36. **(Withdrawn/Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

37. **(Withdrawn/Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

38. **(Withdrawn/Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

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39. **(Withdrawn/Currently Amended)** The method of claim 1, wherein the polyfunctionalized peptide has the structure:

40. (Cancelled)